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[Izmeneniye Soderzhaniya DNK v yadrakh opukholevykh kletok v protesse "progressii" indutsirovannoy hepatomy]

K. M. Pozharisskiy, V. N. Shalumovich and Ya. D. Shaposhnikov

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# CHANGE IN THE DNA CONTENT IN THE NUCLEI OF TUMOR CELLS IN THE PROCESS OF "PROGRESSION" OF INDUCED HEPATOMA

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Biochemical [23,29,33] and cytophotometric [7,10,18,20,21,31,36] investigations have shown that the DNA content of tumors increased in comparison with the homologic normal tissues and that authors who have conducted investigations using cytophotometric methods have detected a direct correlation between the amount of DNA in the nuclei of tumor cells and the degree of the dedifferentiation [5,8]. Thus, Yu. A. Umanskiy et al. [14] showed that with the increase in the duration of effect of a blastomogenic agent on animals, the relative amount of polyploid cells increases in the liver. At the same time, by the aid of biochemical methods, O'Sullivan and Kirby [32] established that the DNA content of hepatic cells increases in the process of carcinogenesis in this organ. The indicated studies were devoted to an investigation of the DNA content in the nuclei of tumor cells or cells in the stage of malignization, and, therefore, the final stage of the investigation was the formed tumor. In the meantime, it is well known that the already formed tumor undergoes changes. In connection with this, the concept of "progression" of tumors appeared [4,24-27,35]. In the accessible literature we did not find studies devoted to a study of the change in DNA content in the process of "progression." Taking into account the fact that the biochemical investigations only provide concepts regarding total changes in the DNA content of the tumor homogenates, and that one can establish individ-

ual tumor cells by the aid of cytophotometry, and consequently, the heterogeneity of the population of tumor cells in this regard, we decided to use the method of cytophotometry of DNA of the nuclei to characterize the "progression" of a tumor.

The material and method. The object of investigation was a strain of hepatocellular carcinoma of the S3NA line obtained in the laboratory. The characteristics of this tumor strain were given in the preceding report [12]. A total of 15 generations of the hepatoma were investigated: transplants from 2 mice of each generation were used for the cytophotometric investigations. Sections of liver of 2 S3NA mouse lines not subject to any special effects were used as the control. The controls were the same age as the mice with tumors. Cytophotometry of the nuclei of 200 cancer cells was carried out in each preparation. Their DNA content was determined on sections 6μ, treated according to Fel'gen. All measurements were made on the MUF-5 microscope with the MIU-2 integrator, using a monochromatic light beam (wave length — 546 millimicrons at probe diameter — 1.4 μ). Depending upon the size and shape of the nuclei, scanning was carried out at 3-7 levels and a mean optic density ($D_{mean}$) was calculated for each of them. This magnitude was determined according to the formual $D_{mean} = \frac{1}{m_{mean}} \cdot \frac{S}{t}$, where S — intensity of absorption (reading of the upper counter of the integrator), t — running length of the scanning beam within limits of the measured object (reading of the lower counter), $m_{mean}$ — conversion coefficient for the mean optical density calculated for the given instrument according to the formula $m_{mean} = \frac{\bar{m}_1}{n}$, where $m_1$ — a different value of the conversion coefficient for different values of optic density, n — number of measurements of mean optical density. Mean optic density of the entire nucleus was determined by means of dividing the sum of mean optic densities of the measured scanning levels by their number.

The DNA content of the nuclei of tumor cells was expressed both in arbitrary units and in ploidy units, since it is widely known that there is a strict correlation between the DNA content and the number of chromosomes. In order to determine the ploidy of the tumor cells, the standard was the DNA content of lymphocyte nuclei on the same

section. As is known, the amount of DNA in the nuclei of lymphocytes corresponds to the diploid chromosome assortment [2,17,22,34,37]. In accordance with this, the coefficient of ploidy (n) was the following value: $n = \frac{S_1 D_1}{SD}$, where $S_1$ — area of section of investigated nucleus, $D_1$ — its mean optic density, and S and D — similar parameters of the lymphocytes. The linear dimensions of the nucleus were determined by the aid of the MOV screw ocularmicrometer — $1\text{-}15^{x}$. The shape of the section of hepatocyte nuclei is circular or oval; the area and volume of the nuclei were determined according to the appropriate formulas [16]. All quantitative readings obtained as the result of the conducted investigation were processed using variation-statistical methods.

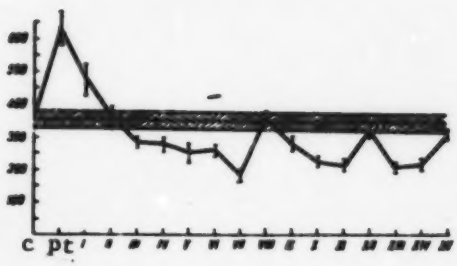


Figure 1. Dynamics of changes in the mean DNA content of the nuclei of malignant hepatocytes in the process of successive inoculations

Along the axis of the abscissa — number of generations (c — control, p.t. — primary tumor). Along the axis of the ordinate — DNA content in arbitrary units. Horizontal line — mean DNA content of the hepatocyte nucleus in the intact mice (control). Shaded region — boundaries of the reliable interval at a 95% level of significance for the control. Vertical lines at the points — boundaries of the reliable interval with a 95% level of significance for each of the periods of the experiment.

Results of the investigation. As the measurements we conducted showed, the mean DNA content of the hepatocyte nuclei in the control male mice of the S3NA line comprised 350 arbitrary units (Figure 1). For primary hepatic tumor cells, the value of this index proved to be much greater (630 arbitrary units). However, in the process of transplanting the hematoma a significant drop in the mean DNA content of transplant nuclei was observed, and the dynamics of these changes in 2 of the investigated sublines of the strain were identical. The smallest value of the DNA content indicator was observed in the 7th generation, where it was much lower than the value of the similar index for cells of the normal liver. However, in succeeding generations fluctuation of the DNA content of the nuclei of tumor cells was observed. This never exceeded the level of the normal hepatocytes.

In order to determine the mechanisms that lie at the basis of the above described dynamics of changes in the DNA content in the process of "progression," an analysis was made of the histograms of ploidy of tumor cell nuclei (Figure 2). The fact that the normal liver has a clearly defined polyploidy attracts attention - the basic mass of the hepatocyte population is made up of di-, tetra-, and octaploidal cells; only a small number of cells comprises 16 n. The somewhat greater degree of polyploidy of the normal hepatic cells in our experiments, in comparison with other observations [1], is apparently explained by differences in the ages of the animals, since it is known that polyploidy of hepatic cells increases proportional to age [2]. The relative number of polyploid cells proved to be particularly large in the primary tumor. It contained very few di- and tetraploid cells; the basic fraction of the population was made up of cells with more than 16 n. Beginning with the second generation, a relative decrease in ploidy of the tumor cells occurred and the basic mass of the tumor was made up by cells within limits of 2 n - 10 n.

On the basis of analyzing the histograms, notwithstanding possible methodological errors, it can still be concluded that the population of tumor cells from transplants of different generations is also characterized by pronounced aneuploidy. The more so, since in the opinion of Hauschka [30], Yu. M. Olenov [9], and Weiss et al.

[38], almost all tumors are characterized by poly- and aneuploidy.

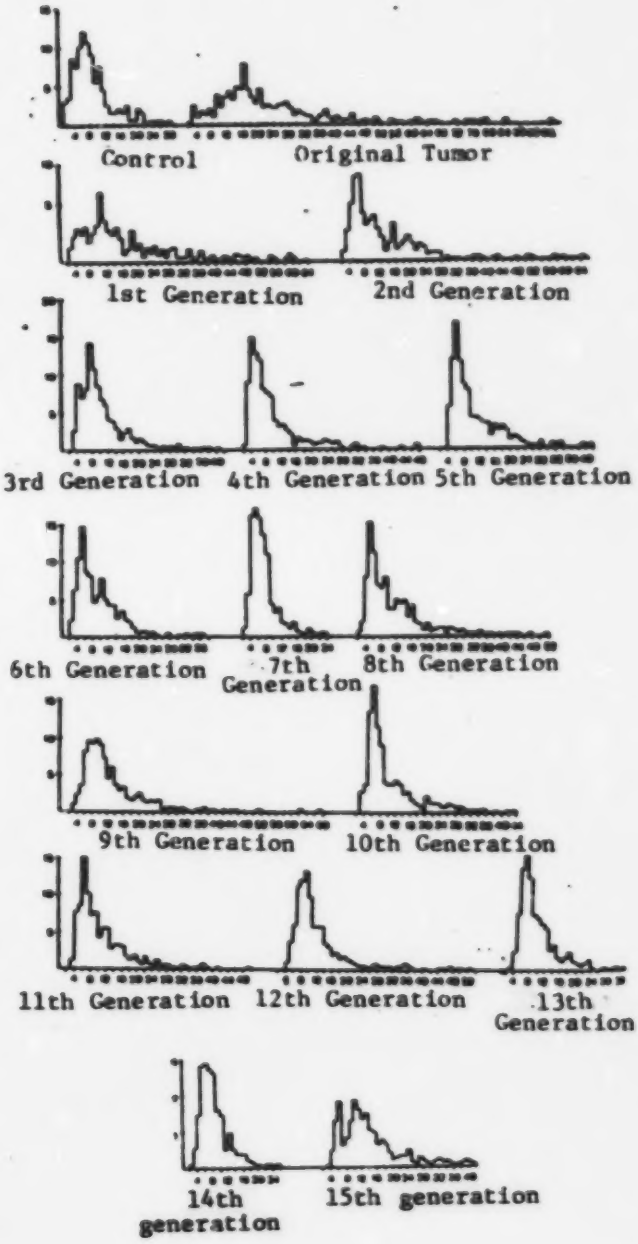


Figure 2. Continuation. Histograms of ploidy of populations of tumor cells of various generations of the hepatoma. Along the axis of the abscissa — DNA content in ploidy units. Along the axis of the ordinate — relative number of nuclei (%).

The $\chi^2$ method [11,13,15], showed that the distribution of cells of different ploidy in all generations of the hepatoma was log-normal. The dynamics of changes in ploidy of tumor cells of different generations of the hepatoma can be characterized by the dispersion indices (Figure 3), which reflect the wavy character with a decreasing amplitude of fluctuations in these changes and an increase in homogeneity of the population of tumor cells with respect to DNA content.

For a more detailed comparative analysis of the quantitative ratios between elements with different ploidy in the transplants of 15 generations and in the initial tumor, all cells were arbitrarily divided into the following classes depending upon DNA content (m): $2n \leq m_1 \leq 4n$; $4n < m_2 \leq 8n$; $8n < m_3 \leq 16n$; $16n < m_4 \leq 32n$ and $32n < m_5 \leq 64n$.

The results of observations grouped in this fashion are shown in Figure 4. From the figure one can see that an almost complete disappearance of cells of the class from 32 n — 64 n occurred proportional to transplantings of the hepatocellular carcinoma (from 15% in the original tumor to 0-5% in subsequent generations). The slight

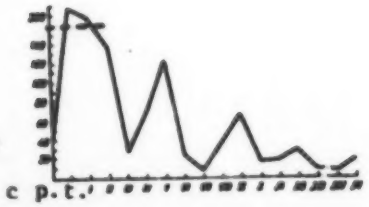


Figure 3. Dynamics of changes in the index of dispersion of ploidy of different generations of the hepatoma. Along the axis of the abscissa — number of generations (c — control, p.t. — primary tumor). Along the axis of the ordinate — dispersion of ploidy (in arbitrary units).

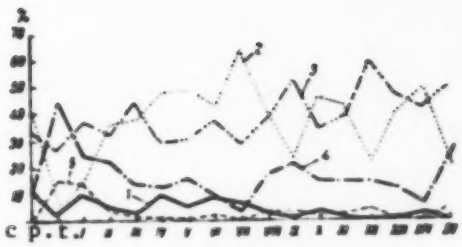

Figure 4. Dynamics of changes in classes of ploidy in different generations of the hepatoma strain. Along the axis of the abscissa — number of generations (c — control, p.t. — primary tumor). Along the axis of the ordinate — number of nuclei (%).

1 — $2n<m_1<4n$; 2 — $4n<m_2<8n$; 3 — $8n<m_3<16n$; 4 — $16n<m_4<32n$; 5 — $32n<m_5<64n$

proportion of tumor elements was comprised by the 2n — 4n class. Hence, the population of tumor cells in the transplants, beginning with the III generation, was basically made up of polyploid elements of 2 classes ($m_2$ and $m_3$).

An analysis of the phase diagrams [28] showed that there is a direct correlation between the dynamics of change in the number of tumor cells with very high ploidy (classes $m_4$ and $m_5$). At the same time, the relative number of cells with high ploidy ($m_4$ and $m_5$), on the one hand, and the relative number of cells of the classes $m_1$ and $m_2$, on the other hand, change in opposite phase, i.e., have an inverse correlation.

An analysis of the histograms of ploidy of the cellular populations of transplants of the tumor of different generations shows that a change in the "stem line" occurs in the process of transplantations. Thus, in the normal liver in our experiments the "stem line" consists of cells with 8 n, while at the same time it changes toward 16 n in the primary hepatic tumor. In the first generation, cells containing DNA in which 12 n is present are most often encountered. Beginning with the second generation, the "stem line" stably lies in the region of 6 n - 10 n.

In addition to the quantitative changes in the DNA content in populations of tumor cells, a change in the distribution of the Fel'gen-positive substance is also noted in the process of subsequent transplantations. As follows from the light absorption curves of the Fel'gen-positive substance, the distribution of substance in the nuclei of cells of the same generation of the tumor strain is extremely variable, but in the process of transplantations the character of curves significantly changes (Figure 5).

Thus, as a rule, the nuclei of cells of the primary hepatic tumor, have 2 absorption maxima — at the beginning and end of the curve with a significant "gap" in the mid region (in a number of cases, even to 0). Occasionally such a curve has a sawtooth character at the midpoint. These observations indicate the localization of DNA predominantly along the periphery of the nucleus, while at the same time its central part is almost entirely free of this substance or contains quite large granules or lumps. The base of these curves is significantly extended, which indicates the large dimension of the nuclei.

This character of the curves is maintained over the course of the first and second, and to a lesser degree, in the third and fourth generations. Subsequently, the curves acquire a more even aspect — they are devoid of even small spikes; the length of their base is maintained. All of this indicates a more uniform distribution of DNA in the nuclei of the tumor cells and a significant decrease in the size of the nuclei themselves proportional to the transplantations. The latter is confirmed by the direct change in volumes of hepatocyte nuclei in the process of "progression"

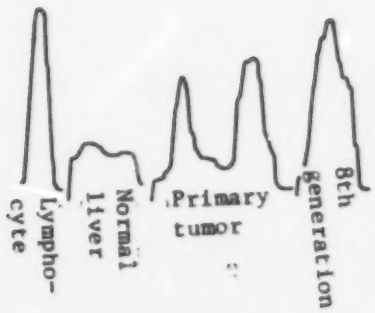


Figure 5. Character of distribution of DNA in nuclei. Absorption curve — Fel'gen-positive substances. Wave length — 546 millimicrons.

of the experimental hepatoma (Figure 6).

Discussion. The obtained results indicate that the population of cells of the preliminarily induced hepatoma is characterized by most pronounced heterogeneity with respect to its containing cells with different classes of ploidy, and therefore, apparently, has an "information maximum" [19] and serves as ground for selecting cells adapted to the new conditions of existence and having properties of autonomy.

Selection appears in a gradual decrease in the relative number of elements with high ploidy and in a change in the "stem line" in the process of tumor transplantation. Obviously, the high degree of ploidy is not maintained by selection [9], and the hyperploid cells are eliminated, while the tumor is characterized by a certain chromosomal assortment determinative for it.

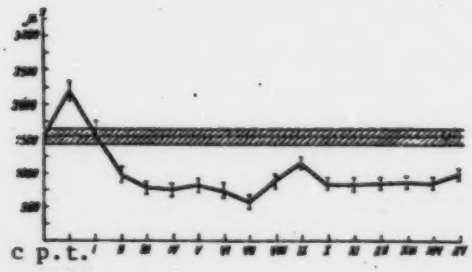


Figure 6. Dynamics of changes in the volume of hepatocyte nuclei in the process of succeeding transplantations.

Along the axis of the abscissa — number of generations (c — control, p.t. — primary tumor). Along the axis of the ordinate — volume of nuclei in μ. Horizontal line — mean volume of hepatocyte nucleus in intact mice (control). Shaded region - boundaries of the reliable interval at a 95% level of significance for the control. Vertical lines at the points — boundaries of the reliable interval at a 95% level of significance for each of the periods of the experiment.

The decrease in cells with high ploidy in the cellular population of the hepatoma had wave-like character, evidence of which are the dispersion indicators. A decrease in this value makes it possible to conclude that as the result of selection in the process of "progression," the population of tumor hepatocytes acquires a constantly growing, homogeneous character; a similar conclusion can be drawn with respect to volumes of nuclei of these cells.

It is considered that the basis of "progression" are multiple changes in the properties of tumor cells [3]. The obtained data make it possible to hypothesize that a great role is placed by selection, besides the indicated mechanism, and this role is similar to that lying at the basis of evolution.

The absence of a clear correlation between the degree of decrease in the mean DNA content in nuclei of the hepatoma and the degree of decrease in their volumes leads to the characteristic hyperchromatose nuclei of the tumors.

There are concepts that morphological characteristics are a relatively stable characteristic of strains of the hepatoma, changing little in the process of transplantation [6]. The results cited in the article, as well as the data obtained earlier on the disruption of the mitotic regime [12], indicate that the quantitative cytological parameters can serve as "progression" criteria.

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